they were less consistent. Smaller seed mass was associated with more poleward distribution in 6 of the 11 dichotomous divergences and with a more tropical distribution in one clade. Larger seed mass was associated with biotic dispersal in 6 of the 11 dichotomous divergences and with abiotic dispersal in one divergence. An analysis across all of the divergences in the tree (based on independent contrasts) also showed that shifts in seed mass have been much more closely associated with shifts in growth form than with shifts in latitude or dispersal syndrome (*35*). Thus, our data are more consistent with Eriksson, Friis, and Lofgren's suggestion (*29*) that changes in seed mass during angiosperm evolution resulted primarily from changes in vegetation structure than with Tiffney's hypothesis (*17*) that changes in dispersal fauna (particularly the radiation of mammals across the Cretaceous-Tertiary boundary) allowed angiosperms to radiate into larger seed masses.

Two of the 11 top-ranking dichotomous divergences in seed mass were not associated with divergences in plant stature. One was the divergence between Juglandaceae and Casuarinaceae/Betulaceae, which was associated with a divergence between biotic and abiotic dispersal. The other was a divergence within Rhizophoraceae, between a small-seeded terrestrial habit and a large-seeded mangrove habit. A shift to a mangrove habit has generally been associated with increases in seed mass. The mangrove habit has evolved in seven families, five of them represented in our database. In four of these (Acanthaceae, Myrsinaceae, Meliaceae, and Rhizophoraceae, but not Combretaceae), mangroves have the largest seeds in the family.

The most consistent pattern we revealed was the association between changes in seed mass and changes in growth form. This result is in line with Charnov's life history theory for mammals (*36*). In Charnov's treatment, offspring size is coordinated with size at adulthood, because larger offspring offset the low survivorship to adulthood that would otherwise be a consequence of longer juvenile periods. This result is also consistent with cross-species studies showing that growth form is the strongest correlate of seed size (*9*, *10*). A recent compilation of data for 2113 species from around the world (*7*) showed a highly significant positive relationship between seed mass and plant height (R^2 = 0.35). Of course, there is still great variation in seed mass for a given plant size. Some of this variation can be attributed to differences in dispersal syndrome, some to biogeography, and more variation is undoubtedly attributable to factors that we have not considered here.

The synthesis of robust phylogenies with global trait data sets holds great promise for elucidating the ecological and evolutionary history of seed plants and of other major groups of organisms.

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A Molecular Phylogeny for Bats Illuminates Biogeography and the Fossil Record

Emma C. Teeling,[1,2*] Mark S. Springer,[3*] Ole Madsen,[4] Paul Bates,[5] Stephen J. O'Brien,[6*] William J. Murphy[1,7]

Bats make up more than 20% of extant mammals, yet their evolutionary history is largely unknown because of a limited fossil record and conflicting or incomplete phylogenies. Here, we present a highly resolved molecular phylogeny for all extant bat families. Our results support the hypothesis that megabats are nested among four major microbat lineages, which originated in the early Eocene [52 to 50 million years ago (Mya)], coincident with a significant global rise in temperature, increase in plant diversity and abundance, and the zenith of Tertiary insect diversity. Our data suggest that bats originated in Laurasia, possibly in North America, and that three of the major microbat lineages are Laurasian in origin, whereas the fourth is Gondwanan. Combining principles of ghost lineage analysis with molecular divergence dates, we estimate that the bat fossil record underestimates (unrepresented basal branch length, UBBL) first occurrences by, on average, 73% and that the sum of missing fossil history is 61%.

Bats are a unique and enigmatic group of mammals that account for ~1,100 species (*1*). They are the only mammals to have achieved true self-powered flight, are found throughout the globe, and play a major ecological role as pollinators and insect predators (*2*). Although

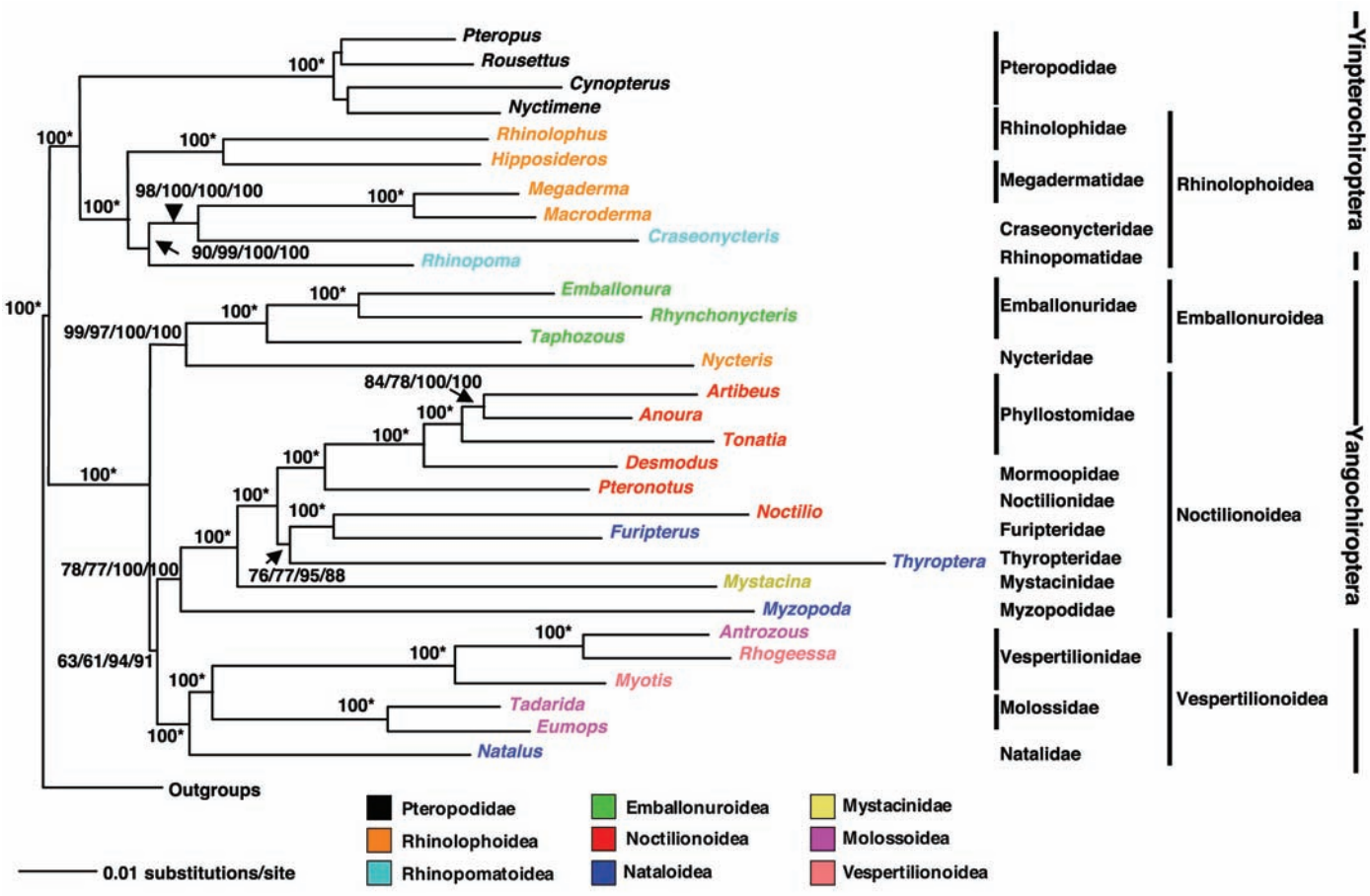

Fig. 1. The maximum likelihood tree (–ln likelihood = 92127.3772) for the concatenated data set under the GTR + Γ + I model of sequence evolution (*11*). Numbers at the nodes are the (ML unconstrained bootstrap values)/(ML constrained bootstrap values)/Bayesian (single-model posterior probabilities shown as percentages)/Bayesian (partitioned model posterior probabilities shown as percentages). 100* signifies clades that received 100% bootstrap support in all analyses and had posterior probabilities of 1.000. The genera are color coded according to the superfamilial groups identified by the most recent morphological phylogenetic study (*4*).

bats originated in the early Eocene, it has been difficult to identify bat species from the fossil record, rendering the chronology of divergence events and biogeography of this order intractable from fossils alone (*3*). Furthermore, the evolutionary history of this order has been obscured by controversial phylogenetic hypotheses. Morphological data traditionally support the monophyly of the order and of the two suborders, Megachiroptera (megabats) and Microchiroptera (microbats), implying a single origin of laryngeal echolocation and flight in bats (*4*). Molecular data support the monophyly of bats and thus a single origin of flight in mammals. However, molecules reveal a sister-taxon relationship between the rhinolophoid microbats and the megabats (Yinpterochiroptera), suggesting either multiple origins of laryngeal echolocation within bats or a single origin of echolocation with subsequent loss in megabats (*5*–*10*). There remains considerable uncertainty in both subordinal and superfamilial classifications within bats, where both morphological and molecular data conflict (*4*, *7*, *10*), and different molecular data sets provide varying support (*8*, *10*).

To discriminate between the competing phylogenetic views, we analyzed 13.7 kb of nuclear sequence data from portions of 17 nuclear genes from representatives of all bat families and four laurasiatherian outgroups [30 bat genera, 4 outgroups; (*11*)]. Phylogenetic analyses with diverse methods resulted in a well-resolved phylogeny, dividing the order into two suborders and four superfamilial groups, rendering microbats paraphyletic (Fig. 1). Both the monophyly of the order Chiroptera and the two suborders Yinpterochiroptera (Rhinolophoidea + Pteropodidae) and Yangochiroptera received 100% bootstrap support (BSS) in all maximum likelihood (ML) analyses and had Bayesian posterior probabilities (BPP) of 1.000 (Fig. 1; table S1). Yangochiroptera is further supported by a 15–base pair (bp) deletion in *BRCA1* and a 7-bp deletion in *PLCB4,* which unites all members of Yangochiroptera, and is absent in all yinpterochiropteran and outgroup taxa (fig. S1). With the inclusion of representatives from all putative microbat families and the addition of 6.1 kb of sequence data from 13 novel nuclear genes, our results strongly support microbat paraphyly. Likewise, some of the superfamilial groupings suggested by previous molecular data are confirmed and extended by this new analysis, and many alternative hypotheses have been refuted (described in table S2).

These data provide a supported resolution for the phylogenetic placement of two enigmatic, monotypic families, Craseonycteridae and Myzopodidae (Fig. 1). *Craseonycteris*

[1]Laboratory of Genomic Diversity, Basic Research Program, SAIC-Frederick, Inc., National Cancer Institute, Frederick, MD 21702, USA. [2]Department of Zoology, University College Dublin, Belfield, Dublin 4, Ireland. [3]Department of Biology, University of California, Riverside, CA 92521, USA. [4]Department of Biochemistry, Radboud University of Nijmegen, Post Office Box 9101, 6500 HB Nijmegen, Netherlands. [5]Harrison Institute, Centre for Systematics and Biodiversity Research, Bowerwood House, 15 St. Botolphs Road, Sevenoaks, Kent TN13 3AQ, UK. [6]The Laboratory of Genomic Diversity, National Cancer Institute, Frederick, MD 21702, USA. [7]Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, USA.

*To whom correspondence should be addressed. E-mail: emma.teeling@ucd.ie; obrien@mail.ncifcrf.gov; mark.springer@ucr.edu

thonglongai, the smallest mammal in the world, is confined to the Kanchanaburi province of western Thailand and southeast Myanmar (*12*, *13*). Our results convincingly place Craseonycteridae within the superfamily Rhinolophoidea (100% BSS, 1.000 BPP) and provide robust support for a sister-group relationship with the megadermatids (100% ML BSS, 1.000 BPP). Further support for the inclusion of *Craseonycteris* within the Rhinolophoidea derives from the possession of pubic nipples, a unique and diagnostic rhinolophoid character (*14*). The phylogenetic position of the Myzopodidae (which consists of the single species *Myzopoda aurita*), endemic to Madagascar (*1*), is also controversial (*4*, *15*). Our data support a basal position for the Myzopodidae within the superfamily Noctilionoidea (78% ML BBS, 1.000 BPP).

A time scale for the evolution of the order Chiroptera based on Bayesian dating analyses (*11*) is depicted in Fig. 2. We estimate that crown group bats last shared a common ancestor about 64 million years ago (Mya) at or following the Cretaceous-Tertiary boundary (fig. S2; table S3) (*11*). This date is also corroborated by a comprehensive eutherian study that primarily used non-chiropteran fossil calibration points (*16*). The four major microbat (echolocating) lineages [Rhinolophoidea, Emballonuroidea, Noctilionoidea, Vespertilionoidea] each originated within a narrow time frame, 52 to 50 Mya, coincident with an approximate 7° rise in mean annual temperature, a significant increase in plant diversity, and the peak of Tertiary insect diversity (*17*–*19*). We suggest that extant microbats diversified in response to an increase in prey diversity and that the varied microbat echolocation and flight strategies may have resulted from differential niche exploitation at that time.

Using this complete interfamilial phylogeny, we examined competing biogeographical hypotheses regarding the origin of bats. The oldest definitive bat fossils are early to middle Eocene, distributed in North America (*Icaronycteris*), Europe (*Hassianycteris, Archaeonycteris*, and *Paleochiropteryx*), and Australia (*Australonycteris*), and they were already specialized for flight and echolocation (*4*, *20*–*22*). They overlapped in range with the modern extant microbat lineages, whose oldest fossil record is from the middle Eocene of Europe (*4*, *23*, *24*), and indeed appear to nest within crown group Chiroptera (*25*). We reanalyzed the morphological data set of Simmons and Geisler (*4*) for extant bat families and extinct Eocene fossils by incorporating the molecular scaffold from Fig. 1 in parsimony analyses (Fig. 3) (*11*). The most parsimonious trees were used to map both current and past geographic distributions in a parsimony framework (Fig. 3) (*11*).

Geographic ancestral reconstructions (*11*) suggest that bats originated in the Laurasian land masses, possibly in North America during the early Paleocene, and fail to support a Gondwanan origin for bats, even with the inclusion of *Australonycteris* in the analyses (Fig. 3; table S4). A Southern Hemisphere origin of modern bats has been suggested [(*26*) and included references], but it is based mainly on current distribution of maximum bat diversity and has been confounded by unreliable phylogenies. Currently, bats are distributed throughout the globe, however, at each taxonomic level bat endemism is high (*1*). All ancestral reconstructions support an Asian origin for the suborder Yinpterochiroptera (Fig. 3; table S4). Since their diversification in the late Paleocene, yinpterochiropterans have had an exclusively Old World distribution (*24*). In contrast, the biogeographic history of Yangochiroptera is more difficult to decipher because of its panglobal distribution (*1*). Our results support a Laurasian, and most likely Asian/European, origin for Yangochiroptera (Fig. 3; table S4). Within this suborder, the emballonurids have an exclusively tropical

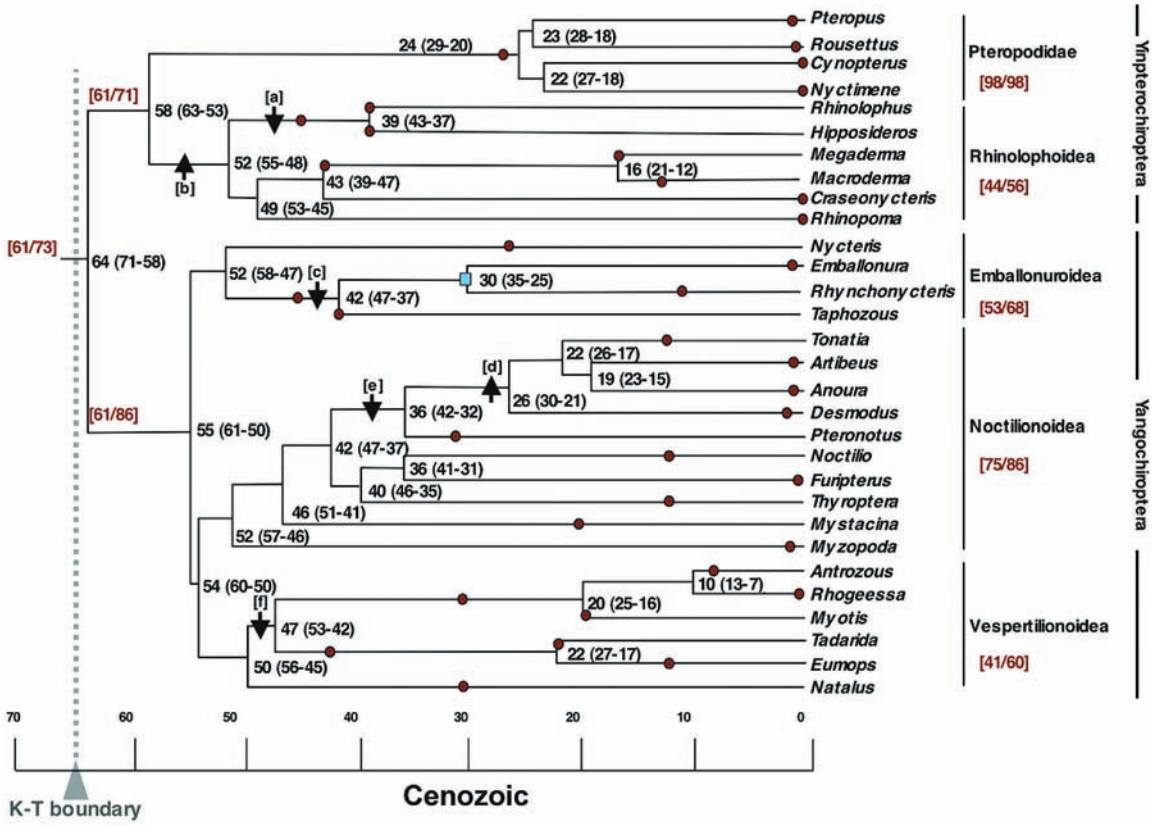

Fig. 2. Molecular time scale for the order Chiroptera based on the *divtime* analyses (*11*), using the ML topology depicted in Fig. 1, six fossil constraints, and a mean prior of 65 Mya for the base of the ingroup root. Numbers at the nodes are the molecular dates in millions of years; values in parentheses are the 95% credibility intervals. Letters along the branches refer to the fossil constraint age (Mya) imposed on that particular node: [a] = 37; [b] = 55; [c] = 37; [d] = 34; [e] = 30; [f] = 37. Maximum constraint is an arrow pointing up; minimum constraint is an arrow pointing down. Red circles indicate the age of the oldest fossil representing that lineage or "off-shoots" from that lineage (table S5). Red numbers in brackets to the left of the slash indicate the percentage sum missing of the fossil record for that clade, (total sum missing per lineage)/(sum age of lineage). Numbers in brackets in red to the right of the slash indicate the average percentage missing of that fossil record for that clade or the average of the percentage missing per lineage (*11*) (table S5). A blue square indicates the time of separation between the New World *Rhynchonycteris* and the Old World *Emballonura*.

distribution on opposite sides of the Atlantic. The oldest fossils are middle Eocene in age from the Messel of Germany (*21*), whereas the oldest New World fossils are Oligocene in age (*3*). Our molecular dates indicate that the split between African and South American emballonurids (i.e., *Emballonura* versus *Rhynchonycteris*) occurred about 30 Mya (Fig. 2; fig. S2; table S3). The arrival of new world monkeys and caviomorph rodents into South America from Africa, possibly via a "vegetational raft" sailing from the Gabon to Brazil or "stepping stones" spanning the Atlantic, is also estimated to have occurred at least 31 to 25 Mya (*27*). We hypothesize that emballonurid bats also arrived to South America via this dispersal route and represent another mammalian lineage that made this journey.

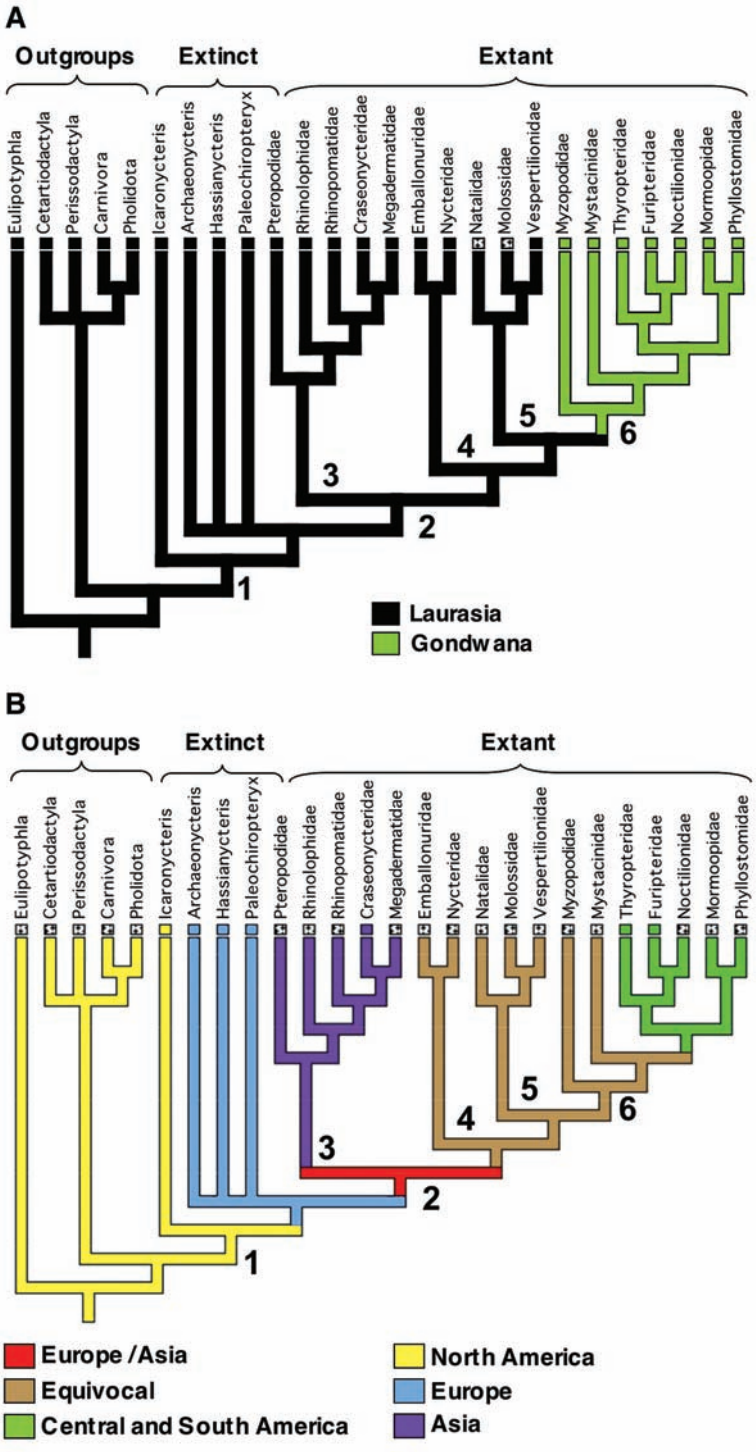

Fig. 3. Biogeographic reconstructions. The topology of the chiropteran taxa is the strict consensus topology of the six most parsimonious trees resulting from the reanalysis of the Simmons and Geisler (*4*) data set with the molecular constraint depicted in Fig. 1. The topology of the outgroup laurasiatherian orders is taken from Murphy *et al.* (*30*). All geographic characters depicted in table S7 were mapped onto each of the most parsimonious trees using accelerated and delayed transformations and the consensus results are shown as follows: (**A**) The earliest occurrences of each lineage in Laurasia or Gondwana, [polymorphic states indicated by a hatched box at tip of branch (*11*); table S4, table S7]. (**B**) Geographic distributions defined by nine character states (*11*) (table S4, table S7). Numbers at the branches identify the following clades: 1, Chiroptera; 2, Yangochiroptera; 3, Yinpterochiroptera; 4, Emballonuroidea; 5, Vespertilionoidea; and 6, Noctilionoidea. Results were considered equivocal if the delayed and accelerated transformations conflicted (table S4).

Living noctilionoids have a disjunct distribution: phyllostomids, mormoopids, noctilionids, furipterids, and thyropterids are mainly confined to the Neotropics; mystacinids are found only in New Zealand; and myzopodids are restricted to Madagascar (*1*). Our ancestral reconstructions suggest that noctilionoids originated in Gondwana, perhaps in South America (Fig. 3; table S4). Their distribution and center of origin are similar to that of the flightless ratite birds (*28*). Molecular dating suggests that the ratites diversified as a result of vicariant speciation due to the break up of Gondwanaland (*28*), whereas our molecular dates estimate the origin of noctilionoids at 52 Mya (Fig. 2), too late to be explained by vicariance. At that time, dispersal was possible between the Gondwanan land masses of South America, Antarctica, and Australia. However, New Zealand, Africa, and Madagascar were already well separated (*29*).

Our molecular dates suggest that there are large gaps in the fossil record for most bat lineages (represented by 58 branches: 30 terminal branches, 28 internal branches; fig. S3), confirming the long held view that the bat fossil record is impoverished. By collating the oldest fossil for every branch on the tree and comparing it with the Bayesian estimated molecular divergence date for that branch, we calculated the unrepresented basal branch length (UBBL) for each lineage. Using this value, we quantified the fraction of each branch underestimated by the fossil record (*11*) (table S5). On average, the fossil record underestimates the origin of 58 bat lineages by 73% (Fig. 2). The four major microbat lineages are missing on average 56 to 86% of fossil history, with the Gondwanan clade (noctilionoids) missing the most (Fig. 2). Megabat lineages are missing a sum total of 98% of their fossil history (table S5). The terminal and internal branches are missing on average 58 and 88% of fossil history, respectively (table S5). With well over half of the Cenozoic history missing for microbat lineages and nearly all of the fossil history missing for megabat lineages, it is not surprising that Paleocene bat ancestors having transitional morphological adaptations for flight and echolocation have never been discovered.

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Interindividual Variation in Posture Allocation: Possible Role in Human Obesity

James A. Levine,* Lorraine M. Lanningham-Foster, Shelly K. McCrady, Alisa C. Krizan, Leslie R. Olson, Paul H. Kane, Michael D. Jensen, Matthew M. Clark

Obesity occurs when energy intake exceeds energy expenditure. Humans expend energy through purposeful exercise and through changes in posture and movement that are associated with the routines of daily life [called non-exercise activity thermogenesis (NEAT)]. To examine NEAT's role in obesity, we recruited 10 lean and 10 mildly obese sedentary volunteers and measured their body postures and movements every half-second for 10 days. Obese individuals were seated, on average, 2 hours longer per day than lean individuals. Posture allocation did not change when the obese individuals lost weight or when lean individuals gained weight, suggesting that it is biologically determined. If obese individuals adopted the NEAT-enhanced behaviors of their lean counterparts, they might expend an additional 350 calories (kcal) per day.

Obesity is epidemic in high-income countries. In the United States alone poor diet and physical inactivity are associated with 400,000 deaths per year (*1*) and obesity-related medical expenditures in 2003 approximated $75 billion (*2*). Obesity is also an emerging problem in middle- and low-income countries, where the health and fiscal costs are likely to be devastating (*3*).

As the impact of obesity on health escalates, so too does the need to understand its pathogenesis. Weight gain and obesity occur when energy intake exceeds energy expenditure. We are interested in a specific component of energy expenditure called NEAT and the role it might play in human obesity. NEAT is distinct from purposeful exercise and includes the energy expenditure of daily activities such as sitting, standing, walking, and talking.

We have previously shown that when humans overeat, activation of NEAT helps to prevent weight gain (*4*). To better understand NEAT and its role in obesity, we separated NEAT into the thermogenesis associated with posture (standing, sitting, and lying) and that associated with movement (ambulation).

Endocrine Research Unit, Mayo Clinic, Rochester, MN 55905, USA.

*To whom correspondence should be addressed. E-mail: Jim@Mayo.edu

To investigate whether the obese state has an effect on NEAT, we first developed and validated a sensitive and reliable technology for measuring the postural allocation of NEAT in human volunteers (*5*, *6*). This physical activity monitoring system uses inclinometers and triaxial accelerometers to capture data on body position and motion 120 times each minute. By combining these measurements with laboratory measures of energy expenditure, we can summate NEAT and define its components (*7*).

To compare body posture and body motion in lean and obese people, we recruited 20 healthy volunteers who were self-proclaimed "couch potatoes." Ten participants (five females and five males) were lean [body mass index (BMI) 23 ± 2 kg/m^2] and 10 participants (five females and five males) were mildly obese (BMI 33 ± 2 kg/m^2) (*8*) (table S1). We deliberately selected mildly obese subjects who were not incapacitated by their obesity and who had no joint problems or other medical complications of obesity. The volunteers agreed to have all of their movements measured for 10 days and to have their total NEAT measured with the use of a stable isotope technique (*9*). They were instructed to continue their usual daily activities and occupations and not to adopt new exercise practices. Over the 10-day period, we collected ~25 million data points on posture and movement for each volunteer.

Our analysis revealed that obese participants were seated for 164 min longer per day than were lean participants (Fig. 1A). Correspondingly, lean participants were upright for 152 min longer per day than obese participants. Sleep times (lying) were almost identical between the groups. Total